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Anatomical studies of *Baccharis grisebachii* Hieron. (Asteraceae). Used in folk medicine of San Juan province, Argentina

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Summary

Baccharis grisebachii Hieron., commonly known as "quilchamali", is a bushy plant that lives in the high mountains of Argentina and southern Bolivia. The infusion or decoction of aerial parts is used in the traditional medicine of San Juan province, Argentina, to treat gastric ulcers, digestive problems, and as antiseptic and wound healing in humans and horses. The aim of this study is to analyze the anatomical characters of *B. grisebachii* for specific identification and quality control. The results show that the leaf blade is ericoid with a dorsiventral mesophyll, and epidermis has a smooth and thick cuticle. The stomata are anomocytic. In both epidermis there are two types of hairs, not glandular and glandular. Adult stems show secondary structures. The root shows 1 - 2 rows of pericyclic cells and an endodermis. *B. grisebachii* shows xeromorphic anatomic characters. The structural characters provide micrographic reference standards, useful for quality control at the crude drug stage.

Estudio anatómico de *Baccharis grisebachii* Hieron. (Asteraceae). Usada en la medicina tradicional de la Provincia de San Juan, Argentina

Resumen

Baccharis grisebachii Hieron. conocida comúnmente como "quilchamali" es una planta arbustiva que vive en las altas montañas de la Argentina y el sur de Bolivia. La infusión o decoción de las partes aéreas es utilizada en la medicina tradicional de la provincia de San Juan, Argentina, para tratar las úlceras gástricas, problemas digestivos y como cicatrizante de heridas y antiséptica en humanos y equinos. El objetivo de este estudio es analizar las características anatómicas de *B. grisebachii* útiles en la identificación y control de calidad de la especie. Los resultados muestran que la hoja es ericoide con mesofilo dorsiventral y tiene una epidermis con una cutícula gruesa y lisa. Los estomas son anomocíticos. En ambas epidermis se encuentran dos tipos de pelos, no glandulares y glandulares. En tallos adultos se hace evidente una estructura secundaria y en la raíz se observan 1-2 hileras de células pericíclicas y la endodermis. *B. grisebachii* muestra caracteres anatómicos xeromórficos. Los caracteres estructurales enunciados proporcionan patrones de referencia micrográficos, útiles para el control de calidad de la droga cruda.

Key words: quilchamali - anatomical studies - epidermis - leaf - stem - root.
Palabras clave: quilchamali - estudios anatómicos - epidermis - hoja - tallo - raíz.

Introduction

Baccharis grisebachii Hieron. (Asteraceae) is widely distributed in the South of Bolivia, the Northwest and West of Argentina between 2,000 and 3,800 m.a.s.l. (Cabrera, 1978; Giuliano, 2000). The populations of *B. grisebachii* in Argentina are located in the Puneña Phytogeographical Region. The dominant vegetation type is the shrubby steppe, with shrubs of half to a meter high, that grow very spread, leaving big spaces of nude soil among them. Some typical species in this area are: *Ephedra breana*, *Fabiana densa*, *Baccharis incarum*, *Adesmia spinosissima*, *Chuquiraga erinacea*, *Lycium fuscum*, *Mulinum spinosum*, *Stipa vaginata*, and *Junellia seriphioides* (Cabrera, 1994).

San Juan province is located in the Central-Western region of Argentina, centred on the intersection of 31° S latitude and 69° W longitude to the Western Andean slopes. The mountains of this range along the San Juan borders are higher than 4,000 m.a.s.l. There are many valleys and desert areas with low rain and snow precipitancy levels. The native flora comprises a large number of species distributed into different ecosystems characterized by particular edaphic and climatic conditions (Feresin and col., 2002). The province has a rich tradition in folk medicine including medicinal plants (Bustos and col., 1996). These species are used as food condiments, infusions or decoctions to treat liver problems, stomach disorders, ulcers and skin infections and domestic pests. The plants are consumed as tea/plants of decoction, isolated or mixed with tea and "yerba mate" (*Ilex paraguariensis*), and are also characterized by a strong scent.

The genus *Baccharis* is an important source of natural medicinal products (Abad and Bermejo, 2007). In the traditional medicine of San Juan province there are at least three species of the genus *Baccharis*, vernacularly known as quilchamalí, which are collected for the retail sale in herbal stores. These species are: *B. grisebachii*, *B. incarum*, and *B. polifolia*. From them, the infusions or decoctions of aerial parts from *B. grisebachii* are used in traditional medicine of San Juan province, Argentina to treat gastric ulcers as a digestive, and as antiseptic and antibiotic for external use. Crushed leaves and flowers are applied as a wound healing poultice to human or horses (Bustos and col., 1996).

During the last years, phytochemical as well as *in vitro* and *in vivo* ethnopharmacological researches have been reported that support the widespread use of extracts and essential oil of *Baccharis grisebachii* in the traditional medicine of Argentina (Feresin and col., 2001; 2002, 2003; Tapia and col., 2004; Hadad and col., 2007). However, anatomical studies of the species *B. grisebachii* Hieron. growing in the Cuyo region, Argentina, have not been reported.

The anatomical studies on different Asteraceae species proved to be incomplete upon bibliographical revision. In this way, Solereder (1908) and Metcalfe and Chalk (1972) have only partially described certain genera. Ramaya (1962a, 1962b) carried out an exhaustive study of certain Asteraceae trichomes. Cortadi and Gattuso (1991) performed the anatomical characterization of *Eupatorium macrocephalum* Less., *E. inulaefolium* Kunth., and *E. subhastatum* Hook. et Arn. There are also several studies regarding xerophytic vegetation of the Puna such as those by Ancibor (1975, 1980, 1982, 2002), and Carmona and Ancibor (1995). Anatomic descriptions of *Baccharis* were reported by Cortadi and col. (1999), Barbosa and col. (2001), Rodríguez and col. (2008) and also included in the taxonomic study of Arizar Espinar (1973), Giuliano (2000, 2001), Rodríguez and col. (2010). In addition, Ancibor (1992) made a short description of the anatomy of *B. grisebachii*, but there is a lack on detailed studies of the anatomy of Quilchamali.

The aim of this work is to accomplish an update of *B.grisebachii* scientific names, synonyms and common names; to provide a brief description of the plant and to undertake the study of the internal anatomy of the vegetative organs employed. This work will therefore provide micrographic reference standards, useful for quality control of the vegetal drug.

Material and Methods

Material used for histology study

Samples for histological studies were collected in Argentina. Prov. San Juan: Dpto. Iglesia. *Loc. Peñasquito. 12/2004, Hadad, M. s/n (MERL 55322). Dpto. Iglesia. *Loc. Agua Negra, 12/2004. Hadad, M, s/n, (MERL 55323). Dpto Iglesia. *Loc. Quebrada de Chita, 12/2004, Hadad, M, s/n, (MERL 55321).

The anatomical study was performed on leaves, petioles, stems and roots, killed and fixed in F.A.A. solution (formaldehyde, ethanol, acetic acid, water, 2:10:1:3). Organs were free-hand cross-sectioned, embedded in paraffin, and cut with a Minot type microtome. Leaves were sectioned in the central part of the lamina. Samples were stained with fast-green safranin (Dizeo de Strittmatter, 1989). Sections were mounted in synthetic balsam. Stems were macerated by conventional methods (Boodle, 1916), and leaves were cleared according to Dizeo de Strittmatter's method (1973). The terminology proposed by Hickey (1973) was used for the description of leaf architecture. Microscopical observations were performed with a Zeiss Axiolab LM and microphotographs were obtained with a MC 80 camera.

Results

Baccharis grisebachii Hieron., *Bol. Acad.Nac. Ci.* 4 (1): 36, 1881. *Baccharis abietina* Kuntze, *Revis. Gen. pl.* 3 (2) 1898; *Baccharis rosmarinifolia* Hook. et Arn. var. *andicola* Hauman, *Anales Soc. Ci. Argent.* 86: 316. 1918. *Common names:* "quinchamal", "romerillo", "tancha".

Description

Dioecious plants. Tomentose shrubs with a height ranging from 0.6 to 2 m. Erect and glabrous branches with brachyblasts. Linear or obovate leaves, obtuse or acuminate apex, entire and revolute margin. Single-ribbed, glabrous stem, whitish or tomentose-grayish in the upper surface, measuring 1.3 - 3 (5) x 0.05 - 0.2 cm. Pedunculated capitula arranged in chorimbiform cymes at brachyblast apex. Filiform, numerous flowers. Yellowish pappus. Male capitula five millimetre length and 4 mm diameter, bellshaped involucre, with three series of acute bracts. Glabrous, five-sided two millimetre length achenes. The plant is widely distributed in the South of Bolivia, the Northwest and West of Argentina, 2,000 and 3,800 m.a.s.l. (Cabrera, 1978, Giuliano, 2000).

Anatomical characters

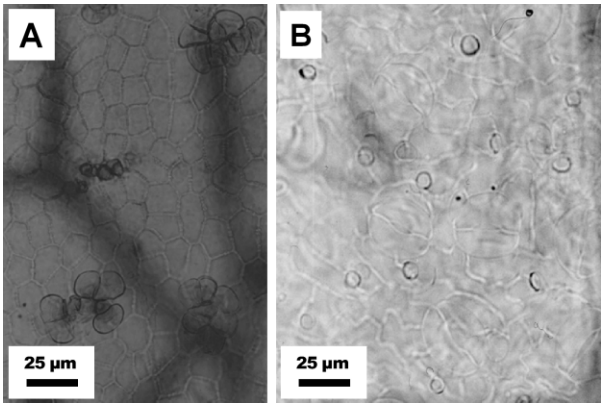
A – Leaf

1- Leaf blade surface view

The analysis of the foliar architecture shows a single ribbed venation and dense vascular pattern.

Epidermis: The adaxial epidermis presents isodiametric cells with right anticlinal walls. The cuticle is thick and smooth without stomata (Figure 1, A). The abaxial epidermis presents anticlinal undulations and stomata in leaf blade depressed zones, and is covered with trichomes. Stomata are anomocytic (Figure 1, B).

Figure 1.- Leaf lamina superficial view of *Baccharis grisebachii*



A. Adaxial epidermis (with glandular trichomes). **B.** Abaxial epidermis (with stomata).

2- Lamina cross-section

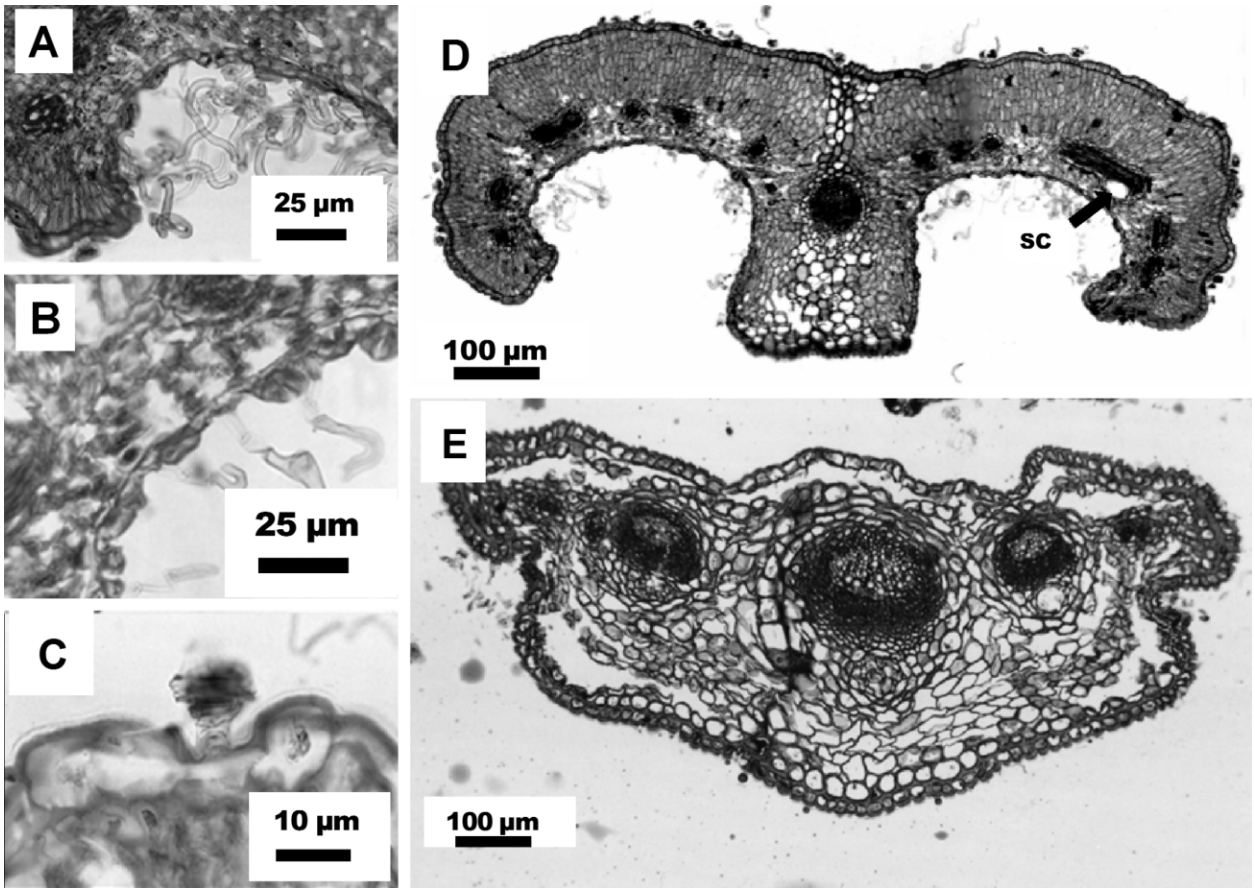
Ericoid leaves. Adaxial and abaxial unistratified epidermis. Stomata at the same level or slightly protruding over epidermis cells. Both epidermis present trichomes that could be classified as type I, non glandular and type II, glandular.

Type I: uniseriate, flexible single hairs, with a stalk and a head, the former with 3 - 5 cylindrical cells, with transverse walls slightly constricted. The longitudinal upper cells have thin transverse walls or slightly thickened, smooth. The head with 3 - 4 cells slightly narrower than those of the stalk, similar to a whip, with thin transverse walls, and slightly thickened, smooth lateral walls (Figure 2, A and B).

Type II: Glandular hairs, with 1-2 celled stalks and 2 cellular heads. The terminal cells present a vesicular cuticle. These hairs could be unique or arranged in nests (Figure 2, C).

Dorsiventral mesophile with 4 - 5 palisade chlorenchyma layers. Spongy parenchyma with 3 - 4 cells with intercellular spaces.

Figure 2.- *Baccharis grisebacchi* leaf cross sections



A-B. Non glandular trichomes. **C**. Elandular trichomes. **D.** Ericoid leaf. **E.** Petiole cross-section, schizogenous cavities (sc).

Prominent middle vein in the abxial face, reinforced by lamina-like collenchymas. The collateral vascular bundles in a variable number, surrounded by a parenchymatous sheath (Figure 2, D). Associated with the vascular bundles, schizogenous cavities composed of 1 - 2 layers of thick-walled cells, followed by an inner 1-stratified epithelium (Figure 2, D).

3- Petiole cross-section

The petiole cross-section presents a flat-convex contour. The epidermis is un-stratified with glabrous five-sided papillomatous cells and with a laminar collenchyma composed of 2 - 3 cell layers. There is a central, collateral middle nerve with 2 - 3 smaller vascular bundles at each side. Schizogenous cavities could be observed in the parenchyma (Figure 2, E).

B- Stem

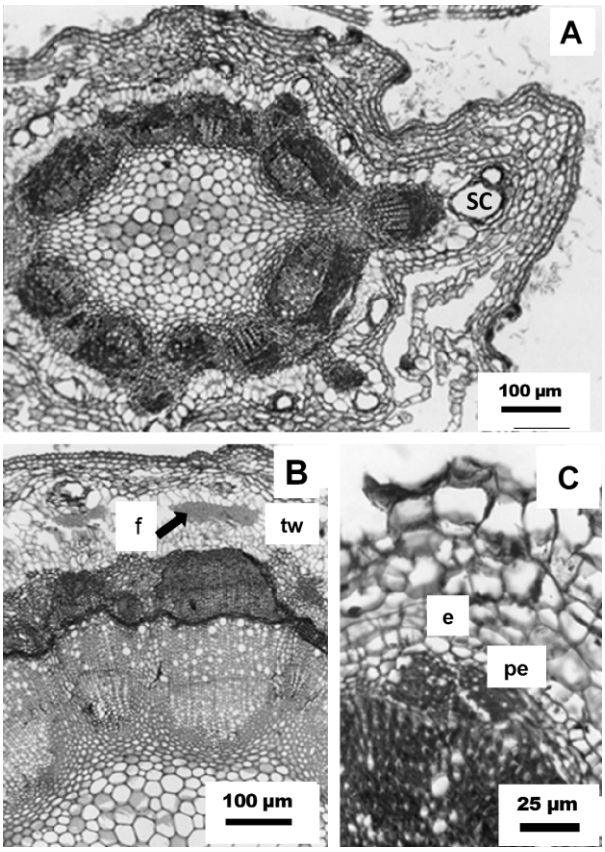
Primary structure

The cross section through the mid zone of the internodes is circular with 6 ribs. There is a mono stratified epidermis of papillomatous cells. The external cortex is made up of 2 - 3 cell layers, laminar collenchyma and parenchyma; the inner cortex is formed by 2 - 3 large cell layers with very thin walls, constituting a water storage parenchyma. Between the external and internal bark schizogenous cavities are observed (Figure 3, A).

Secondary structure.

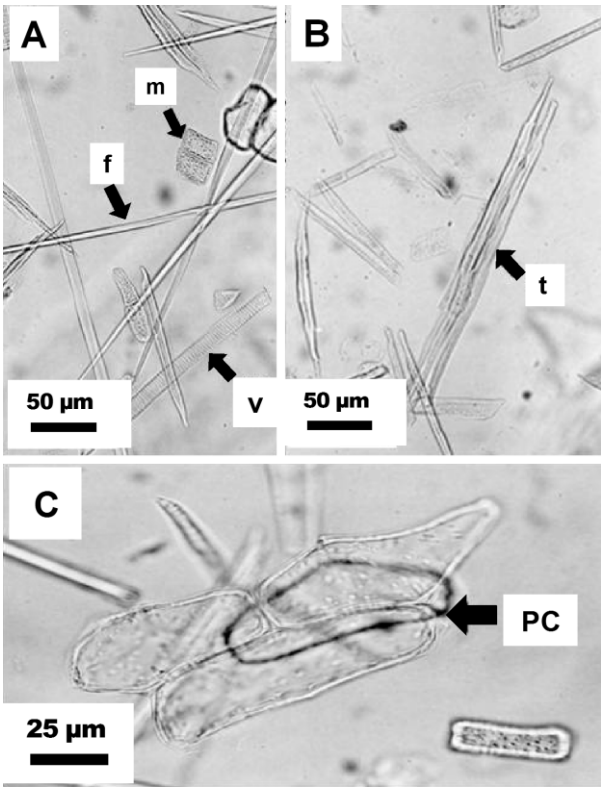
Adult stems evidence a secondary structure of vascular tissues, without peridermis. The inner cortical parenchyma, outside the water storage parenchyma, shows fibre caps. The vascular bundles in

Figure 3.- *Baccharis grisebachii* stem and root cross sections



A. Stem: primary structure. **B**. Stem: secondary structure. **C.** Root: endodermis (e), fibres (f), pericíclic cells (p), schizogenous cavities (sc), thin-walled cell layer (tw).

Figure 4.- *Baccharis grisebachii* stem maceration



Macerated elements of the stem: vessels (v); fibre (f), macrosclereids (m), parenchyma cells (pc), tracheids (t).

the secondary structure are accompanied by fibres close to the pith. The phloem fibres are abundant among the vascular elements. The pith parenchyma has thin cell walls, without intercellular spaces (Figure 3, B).

The dissociated elements of the stem show long and narrow vessels members with simple perforation plates and helical thickenings of 168 x 19 µm, fibres of 844 µm, macroesclereids (Figure 4, A), tracheids of 288 µm (Figure 4, B) and thin-walled parenchyma cells (Figure 4, C).

C- Root

A diarc root structure initially, shows surface suber when mature. The centre is occupied by xylem, displaying a secondary phloem, and a visible cambium, as well as 1 - 2 pericycle-cell layers and endodermis. The cortical parenchyma is narrow (Figure 3, C).

Discussion and Conclusions

The ericoid leaf, the stomata in abaxial epidermis and the water-storage parenchyma in *B. grisebachii* are adaptations to the extreme aridity conditions as observed by Vilela (1993) in *Prosopis nigra*. Mesophyll structure is dorsiventral with 4 - 5 layers of collenchyma unlike another species of the genus that show an isobilateral structure as *B. obovata* (Molares and col., 2009). Anomocytic stomata occurrence in *B. grisebachii* is coincident with the reports to the Asteraceae family (Metcalfe

and Chalk, 1972) and Ariza Espinar (1969) for this species. Rodriguez and col. (2010) showed this same stomata type in *B. articulate*, *B. gaudichaudiana*, and *B. trimera*. Although stomata are at the same level or slightly raised over epidermis cells, this character is not representative of xerophytes plants (Ragonese,1990). However, the transpiration control is given by a thick piliferous coating. The single hairs decrease the air movement in the leaf surface, retaining the water vapor that diffuses from inside to outside. Glandular hairs allow water control losses through transpiration excreting essential oils, this resulting in a thick layer of air on the leaf that prevents air loss vapour (Carmona and Ancibor, 1995; Molares and col., 2009). These types of glandular hairs were observed in other species of the Asteraceae family as *B. triangularis* Hauman (Petenatti and col., 2007), *B. crispa* (Ariza Espinar, 1973; Cortadi et al., 1999), *B. trimera* (Cortadi et al., 1999).

In conclusion, *B. grisebachii* shows xeromorphic anatomic characters that allow it to live in xeric environments. However, the natural drug, whether complete or fragmented, can be identified by means of structural characters which, in order to provide micrographic reference standards, are useful for quality control.

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References

Abad, M. J.; Bermejo P. (2007). "*Baccharis* (Compositae): a review update". *ARKIVOC* (VII): 76-96.

Ancibor, E. (1975). "Estudio anatómico de la vegetación de la Puna de Jujuy I. Anatomía de *Polylepis tomentella* (Rosaceae)". *Darwiniana* 19: 73-385.

Ancibor, E. (1980). "Estudio de la vegetación de la Puna de Jujuy II. Anatomía de las plantas en cojín". *Boletín de la Sociedad Argentina de Botánica* 19(1-2): 157-202.

Ancibor, E. (1982). "Estudio anatómico de la vegetación de la Puna de Jujuy IV. Anatomía de los subarbustos". *Physis Secciones* 41(1009): 107-114.

Ancibor, E. (1992). "Anatomía ecológica de la vegetación de la Puna de Mendoza I. Anatomía foliar". *Parodiana* 7(1-2): 63-76.

Ariza Espinar, L. (1969). "Las especies de *Baccharis* (Compositae) de Argentina Central". Tesis Doctoral. Buenos Aires, Universidad Nacional de Bs. As., Facultad de Farmacia y Bioquímica.

Ariza Espinar, L. (1973). "Las especies de *Baccharis* (Compositae) de Argentina Central". *Boletín de la Academia Nacional de Ciencias* (Córdoba) 50: 175-305.

Barbosa, G.; Bonzani, N.; Filippa E.; Luján M.; Morero R.; Bugatti M.; Decolatti N.; Ariza Espinar, L. (2001). *Atlas histo-morfológico de plantas de interés medicinal de uso corriente en Argentina.* Museo Botánico Córdoba. Serie Especial I. Ed. Graphyon, Córdoba: 8-11.

Boodle, L.A. (1916). "A method of macerating fibres". Roy. Bot. Gard. Kew. *Bull. Misc. Inform*. 5: 108-110.

Bustos, D.A.; Tapia, A.A.; Feresin, G.E; Ariza Espinar, L. (1996). "Ethnopharmacobotanical survey of Bauchazeta district, San Juan Province Argentina". *Fitoterapia* LXVII, Nº 5: 411-415.

Cabrera, A. (1978). "Flora de la Provincia de Jujuy Parte X" *Compositae Colección Científica* INTA Buenos Aires: 234-235.

Carmona, C.S.; Ancibor, E. (1995). "Anatomía ecológica de las especies de *Acantholippia* (Verbenaceae)". *Boletín de la Sociedad Argentina de Botánica* 31(1-2): 3-12.

Cortadi, A.A.; Gattuso, M.A. (1991). "Caracterización anatómica e histoquímica de *Eupatorium macrocephalum* Less., *E. inulaefolium* H.B.K. y *E. subhastatum* Hook et Arn. (Asteraceae)". *Dominguezia* 11(1): 32-42.

Cortadi, A.; Di Sapio, O.; Mc Cargo, J.; Scandizzi, A.; Gattuso, S.; Gattuso, M. (1999). "Anatomical studies of *Baccharis articulata*, *Baccharis crispa* and *Baccharis trimera*, 'carquejas' used in folk medicine". *Pharmaceutical Biology* 37(5): 357-365.

Dizeo de Strittmatter, C. (1973). "Nueva técnica de diafanización". *Boletín de la Sociedad Argentina de Botánica* 15: 126-129.

Dizeo de Strittmatter, C. (1979). "Modificación de una técnica de coloración safranina- fast-green". *Boletín de la Sociedad Argentina de Botánica* 18(3 - 4): 121-122.

Feresin, G.E.; Tapia, A.; Lopez, S.N.; Zacchino S. (2001). "Antimicrobial activity of plants used in traditional medicine of San Juan province, Argentina". *Journal of Ethnopharmacology* 78(1): 100-107.

Feresin, G.; Tapia, A.; Gutierrez Ravelo, A.; Delporte, C.; Backhouse Erazo N.; Schmeda Hirschmann G. (2002). "Free radical sacavengers, anti-inflammatory and analgesic activity of *Acaena magellanica*". *Journal of Pharmacy and Pharmacology* 54: 835-844.

Feresin, G.E. (2002). "Actividad biológica y fitoquímica de especies vegetales de uso medicinal en la Provincia de San Juan, Argentina". Tesis doctoral. Universidad Nacional de San Luis, Argentina.

Feresin, G.E.; Tapia, A.; Gimenez, A.; Ravelo, A. G.; Zacchino, S.; Sortino, M.; Schmeda-Hirschmann, G. (2003). "Constituents of the Argentinian medicinal plant *Baccharis grisebachii* and their antimicrobial activity". *Journal of Ethnopharmacology* 89(1): 73-80.

Giulano, D.A. 2000. "Asteraceae, parte 15, en Flora Fanerogámica Argentina". *PROFLORA* 66: 3-67.

Giulano, D.A. (2001). "Clasificación infragenérica de las especies argentinas de *Baccharis* (Asteraceae, Astereae)". *Darwiniana* 39: 138-154.

Hadad, M.; Zydgalo, J.A.; Lima, B.; Derita, M.; Feresin, G. Zacchino, S.A.; Tapia, A. (2007). "Chemical composition and antimicrobial activity of essential oil from *Baccharis grisebachii* Hieron (Asteraceae)". *Journal of the Chilean Chemical Society* 52(2): 1186-1189.

Hickey, J. (1973). "Classification of the architecture of Dicotyledons leaves". *Am. J. Bot.* 60: 17-33, traducida por Zardini, E. (1979). *Boletín de la Sociedad Argentina de Botánica* 16(1-2): 1-26.

Metcalfe, C.R.; Chalk, L. (1957). *Anatomy of Dicotyledons*. Vol. II. Clarendon Press, Oxford: 782-804.

Metcalfe C.R.; Chalk, R. (1972) *Anatomy of the Dicotyledons*. Vol I, 2nd ed. Systematic Anatomy of the Leaf and Stem. Clarendon, Oxford London.

Molares, S.; Gonzalez S. B.; Ladio A.; Agueda Castro, M. (2009). "Etnobotánica, anatomía y caracterización físico-química del aceite esencial de *Baccharis obovata*" Hook. *et* Arn. (Asteraceae: Astereae). *Acta Botanica Brasilica* 23(2): 578-589.

Petenatti, E.M.; Petenatti, M. E.; Cifuente, D. A.; Gianello, J. C.; Giordano, O.S.; Tonn, C. E.; Del Vitto, L. A. (2007). "Medicamentos herbarios en el centro-oeste Argentino. VI. Caracterización y control de calidad de dos especies de 'Carquejas': *Baccharis sagittalis* y *B. triangularis* (Asteraceae)". *Latin American Journal of Pharmacy* 26: 201-208.

Ragonese, A.M. (1990). "Caracteres xeromorfos foliares de *Nassuvia lagascae* (Compositae)". *Darwiniana* 30(1-4): 1-10.

Ramayya, W. (1962a). "Studies on the trichomes of some Compositae I, General Structure". *Bulletin of the Botanical Survey of India* 4(1-4): 177-188.

Ramayya, W. (1962 b). "Studies on the trichomes of some Compositae II Phylogeny and Classification". *Bulletin of the Botanical Survey of India* 4(1-4): 189-192.

Rodríguez, M.; Gattuso, S.; Gattuso, M. (2008). "*Baccharis crispa* y *Baccharis trimera* (Asteraceae): Revisión y Nuevos Aportes para su Normalización Micrográfica". *Latin American Journal of Pharmacy* 27(3): 387-97.

Rodríguez, M. V.; Martínez, M. L.; Cortadi, A. A.; Bandoni, A.; Giuliano, D. A.; Gattuso, S.; Gattuso, M. (2010). "Characterization of three sect. Caulopterae species (*Baccharis*- Asteraceae) inferred from morphoanatomy, polypeptide profile and spectrophotometry data". *Plant Systematics and Evolution* 286: 175-190.

Solereder, H. (1908). *Anatomy of the Dicotyledons*. Vol. I. Clarendon Press, Oxford: 456-469.

Tapia, A.; Rodriguez J.; Theoduloz, C.; Lopez, S.; Feresin, G. E.; Shmeda-Hirscmann, G. (2004). "Free radical Scavengers and Antioxidants from *Baccharis grisebachii*". *Journal of Ethnopharmacology* 95: 155-161.

Vilela, A.E. (1993). "Anatomía foliar de *Prosopis* (Leguminosae-Mimosoideae): Estrategias adaptativas de diferentes ambientes en *Prosopis nigra*". *Darwiniana* 32(1-4): 99-107.